Report for 2002NH1B: Linking Lakes with the Landscape: The Fate of Terrestrial Organic Matter in Planktonic Food Webs

- Articles in Refereed Scientific Journals:
  - Lennon, J.T. Terrestrial carbon input drives CO2 output from lake ecosystems. In review, Oecologia.
- unclassified:
  - Lennon, J.T. Sources of terrestrial-derived subsidies affects aquatic bacterial metabolism. In preparation for submission to Microbial Ecology.

Report Follows:

WRRC FY 2002 Annual Progress Report

LINKING LAKES WITH THE LANDSCAPE: THE FATE OF TERRESTRIAL ORGANIC MATTER IN PLANKTONIC FOOD WEBS

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PROBLEMS AND OBJECTIVES

We are evaluating how terrestrially-derived dissolved organic matter (DOM) influences the functioning of lake ecosystems. Terrestrially-derived DOM is commonly the largest carbon pool in lakes. As such, terrestrial DOM represents a major source of potential energy for aquatic food webs that may subsidize higher trophic levels (including zooplankton and fish) and determine whether lake ecosystems act as sources or sinks of CO_2. Our project addresses three main factors that influence terrestrial carbon flow in lakes.

Objective 1: Determine whether the energetic importance of terrestrial DOM in lakes is ultimately determined by bacterial metabolism. In this study, we define bacterial metabolism as biomass production and respiration. We hypothesized that bacterial biomass production and respiration are affected by the quantity and quality of terrestrial DOM. Specifically, we predicted that bacterial metabolism is higher on DOM sources with low C:N and C:P ratios.

Objective 2: Quantify the extent to which carbon flow in lakes is influenced by food web structure. One important feature of lake food webs is that not all members of the plankton community are capable of consuming DOM-subsidized bacteria. We hypothesized that food web structure affects whether higher trophic levels benefit from inputs of terrestrial DOM. We predicted that food webs dominated by cladoceran zooplankton, which are capable of feeding on bacteria, would be more efficient at transferring terrestrial carbon to higher trophic levels.

Objective 3: Use carbon stable isotopes ($\delta^{13}C$) in particulate organic matter (POM) and zooplankton to elucidate patterns of carbon and nutrient cycling along a terrestrial DOM gradient in New England lakes. We predicted that carbon stable isotope ratios in zooplankton and particulate organic matter would become more negative along the DOM gradient, reflecting a terrestrial $\delta^{13}C$ signal. We also predicted that cladoceran zooplankton would have lower $\delta^{13}C$ values than copepod zooplankton because they can feed on terrestrial subsidized bacteria.

METHODS

Objective 1*: DOM characteristics and bacterial metabolism*— We measured bacterial productivity (BP) and bacterial respiration (BR) on six different sources of terrestrial-derived DOM. We collected soils from the organic (Oa/A) horizons in near-monoculture stands of six of the most common trees in New England forests (pine, maple, hemlock, beech, oak, birch). We dried these soil samples and then extracted the organic matter in 1L 0.1N NaOH. We removed particulate material (>0.7 μm) from the leachates via serial filtration, dialyzed each leachate (500

D) in a distilled water buffer to reduce concentrations of salts and inorganic nutrients, and then gamma-irradiated the leachates to kill soil-associated microbes.

We then characterized a suite of chemical properties in each leachate: dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN), polyphenolic compounds, humic acids, protein content, and high molecular weight DOC fractions. In addition, we determined the concentrations of 15 major elements using inductively coupled plasma (ICP) atomic emission spectroscopy (AES).

We used regrowth experiments to quantify bacterial production and bacterial respiration in response to different leachates along a DOC gradient (3-17 mg/L). Each experimental unit was filled with 450 mL of 0.22 μm-filtered lake water and 50 mL of 2.7 μm-filtered (Whatman GF/D filtered) lake water containing bacteria, then concentrated terrestrial leachate was added to create the target DOC concentration. We estimated bacterial productivity (BP) at 36 h by measuring the uptake and incorporation of ^{3}H radiolabeled leucine into bacterial protein. Bacterial respiration (BR) was estimated as the changes in dissolved oxygen concentrations in dark bottles between 24 and 48 h.

Objective 2: ***Food web structure and terrestrial carbon flow*****—** We are using simulation models to address Objective 2. We are currently constructing a nonlinear deterministic simulation model in MatLab to assess how terrestrially-derived DOM could influence the energetics of lake food webs. Differential equations for state variables have been chosen from the primary literature and compartment sizes will be tracked using carbon as the currency. Bacteria and phytoplankton have logistic growth and all consumer-resource interactions are described with Type II ratio-dependent functional responses. Dynamics of the state variables will be solved using adaptive step size Runge-Kutta integration. Once the model is constructed, we conduct two types of sensitivity analyses: (1) independently manipulating the model parameters and (2) conducting Monte Carlo cross-factor simulations using parameter distributions estimated from the literature. After we establish that the model is well-behaved, we will run the model over a range of realistic DOM loading rates for two different food webs (daphniid- vs. copepod-dominated) across a gradient of lake trophic states.

Objective 3: ***Carbon stable isotope ratios along DOM gradients***— During summer 2002, we sampled 37 lakes in VT, NH, CT, and ME for $\delta^{13}C$ in POM and crustacean zooplankton. At each lake, we recorded physical-chemical features of the lake water (i.e., pH, O_2, conductivity), then took depth-integrated water samples from the epilimnion of each lake. Samples for POM were filtered onto precombusted glass fiber filters in the field, and samples for DOC, TN,TP, and chlorophyll *a* were brought back to the laboratory. We also took depth integrated zooplankton samples (>80 μm) and separated animals into two functional groups (large cladocerans and large copepods) before filtering them onto precombusted glass fiber filters. $^{13}C/^{12}C$ ratios were obtained with a Finnigan MAT 252 Isotope Ratio Mass Spectrometer at the UC Davis Stable Isotope Facility. We measured DOC on a Tekmar-Dohrmann TIC/TOC analyzer after H_2SO_4 digestion. We measured TN and TP spectrophotometrically after persulfate digestion and determined chlorophyll *a* with methanol extraction on a Turner Designs TD700 fluorometer outfitted for Welshmeyer's method.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Objective 1*: DOM characteristics and bacterial metabolism*— The chemical composition of the DOM sources (leachates) were significantly different from one another. One-way ANOVA revealed that there were significant differences among the leachates for all chemical attributes. Multivariate principal components analysis (PCA) also indicated that the DOM sources had different chemical compositions.

BP and BR responded strongly to the DOM treatments. A multiple regression model (using DOC and indicator variables for leachate type as the predictors) explained 87% of the variation in BP ($R^2 = 0.88$, $R^2adj = 0.85$, $F_{11,71} = 37.8$, $P < 0.0001$). This analysis revealed that differences in DOM sources explained much of the variability in BP. BP was highest when bacteria were grown on beech and oak DOM. Multiple regression explained 67% percent of the variation in BR ($R^2 = 0.67$, $R^2adj = 0.61$, $F_{11,70} = 10.9$, $P < 0.0001$), but was less affected by the different DOM sources as evidenced by overlapping confidence intervals for the slopes provided by the indicator variables (see Fig. 1).

As predicted, carbon:nutrient ratios were important in explaining variation in bacterial metabolism. Carbon specific rates of BP (i.e., the slope of the DOC-BP relationships) increased exponentially with the phosphorus content of the DOM source.

Together, these results suggest that sources of DOM vary in their chemical composition and that this variability can have a large effect on bacterial metabolism. These differences may influence the degree to which higher trophic levels are subsidized by terrestrial DOM. Work completed as part of this Objective is currently being written up for submission to *Microbial Ecology* during summer 2003.

Objective 2: *Food web structure and terrestrial carbon flow*— We have nearly completed our literature search for parameters needed in the food web model, and have programmed the microbial portion of the food web (bacterial carbon uptake and growth). Analyses of this sub-model indicate that the rate and magnitude at which carbon becomes available to bacteria have considerable effects on bacterial biomass, and that further simulations will need to take the temporal dynamics of carbon inputs into account. Our next step is to integrate the microbial part of this model into a more traditional plankton food web.

Objective 3: *Carbon stable isotope ratios along DOC gradients*—We obtained isotope samples from 37 of the 50 lakes visited in 2002. DOC concentrations in the lakes ranged from 3–15 mg/L. $\delta^{13}C$-POM declined over the DOC gradient from -24 to -36 ‰. Similarly, $\delta^{13}C$-zooplankton declined over the DOC gradient from -27 to -40 ‰ (see Fig. 2). A multiple regression model explained 63% of the variation in plankton $\delta^{13}C$ ($R^2 = 0.63$, $P < 0.0001$, n = 73). A paired t-test revealed that cladoceran vs. copepod zooplankton had similar $\delta^{13}C$ values ($P > 0.05$).

Published isotopic values of the POM and zooplankton are commonly less than terrestrial organic matter (-28‰). This observation, together with the relationship in Fig. 2, suggests that there is a progressively larger input of isotopically light carbon along the DOM gradient. We hypothesize that methane (CH_4) becomes more important for lake carbon cycling as terrestrial DOM inputs increase. Terrestrial DOM colors lake water and attenuates solar radiation, promoting anoxic conditions in the hypolimnia, which in turn favor methanogenic bacterial communities. Methanogenic bacteria convert H_2 and CO_2 to CH_4 and H_2O. CH_4 is then used by methanotrophic bacteria, creating isotopically light CO_2. This summer we plan to continue our lake survey, adding some new measurements to test the importance of CH_4 cycling in terrestrially subsidized lakes. We will analyze hypolimnetic CH_4 concentration, hypolimnetic δ^{13}C-DIC, and bacterial community structure using fluorescence in-situ hybridization (FISH). If CH_4 cycling becomes more important in DOM-rich lakes, and is responsible for the pattern in Fig. 2, then we should see the following: 1) hypolimnetic CH_4 increases with DOC, 2) ^{13}C-DIC becomes more negative with DOC, and 3) methanogenic and methanotrophic bacterial increase with DOC.

OTHER ACCOMPLISHMENTS

Students involved— To date, three students have been involved in this project. Jay Lennon is a Ph.D. student. This grant funds his dissertation research. Liza Pfaff and Nira Salant were Dartmouth undergraduate students employed by this grant; Pfaff worked on the project from April to December, 2002, and Salant worked from October to December, 2002.

Papers—

Lennon, J.T. Terrestrial carbon input drives CO2 output from lake ecosystems. In review. Oecologia.

Lennon, J.T. Sources of terrestrial-derived subsidies affects aquatic bacterial metabolism. In preparation for submission to Microbial Ecology in July 2003.

Presentations—

Lennon, J.T. June 10-14, 2002. Experimental evidence that terrestrial organic matter modifies plankton metabolism. American Society of Limnology and Oceanography, Victoria, British Columbia, oral presentation.

Lennon, J.T. October 16, 2002. Ecology of subsidies: role of terrestrial carbon in aquatic ecosystems. Colby-Sawyer College. Invited seminar.

Lennon, J.T. April 29, 2003. Terrestrial subsidies in aquatic ecosystems: is carbon flow to higher trophic levels regulated by microbial metabolism? Cary Conference, Institute of Ecosystem Studies, poster presentation.

Lennon, J.T. and Pfaff, L.E. August 3-8, 2003. Microbial constraints on the flow of terrestrial subsidies in lake ecosystems. Ecological Society of America, Savannah, GA, oral presentation.

Lennon, J.T. November 4-7, 2003. Trophic state and plankton nutrition along a terrestrial DOM gradient in New England lakes. North American Lake Management Society. Mashantucket, Connecticut, oral presentation.

Special session organizer—

Lennon co-organized a special session on "Ecological implications of terrestrial inputs into lakes and ponds" for the 2002 American Society of Limnology and Oceanography national meeting.

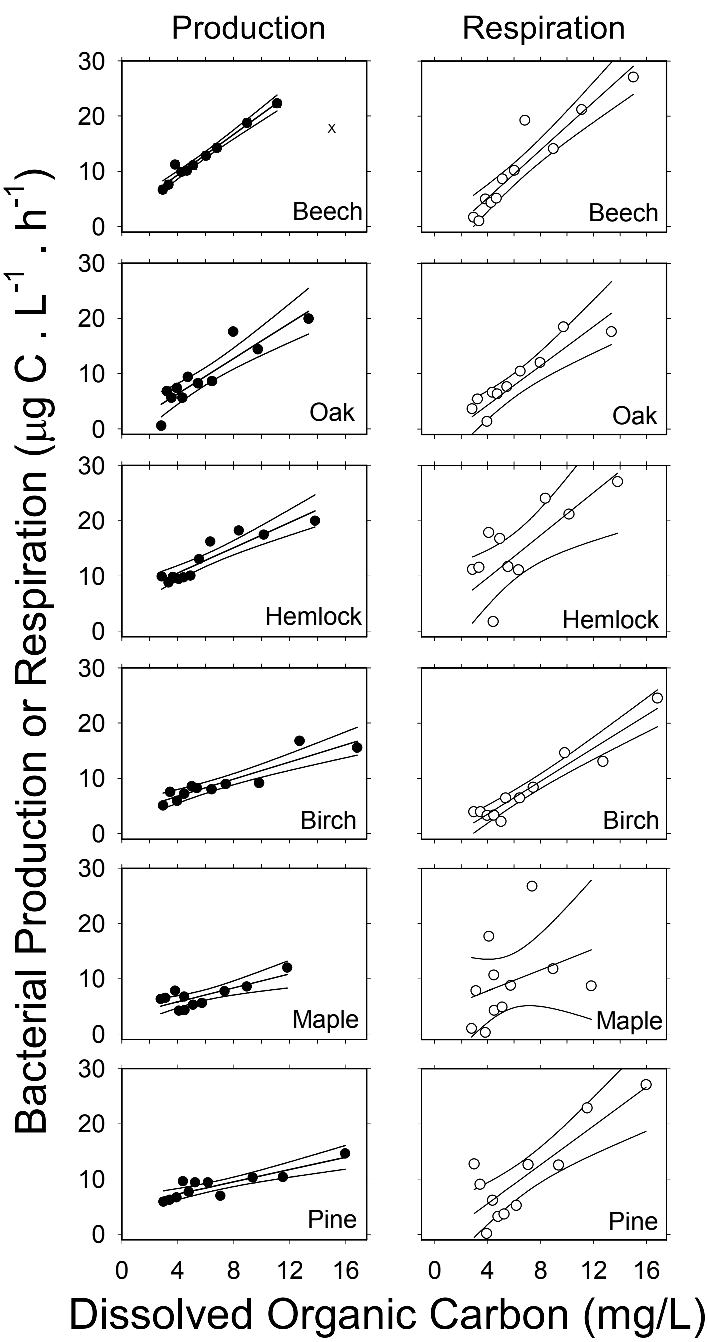

Figure 1. Bacterial production (●) and bacterial respiration (○) in response to varying quantities and sources of terrestrial-derived DOM. Bacterial production and bacterial respiration increased with dissolved organic carbon (DOC). Bacterial production was affected by different DOM sources (slopes are ranked highest to lowest along the vertical panels); bacterial production was greatest on beech and oak DOM sources. Bacterial respiration was not affected by DOM source.

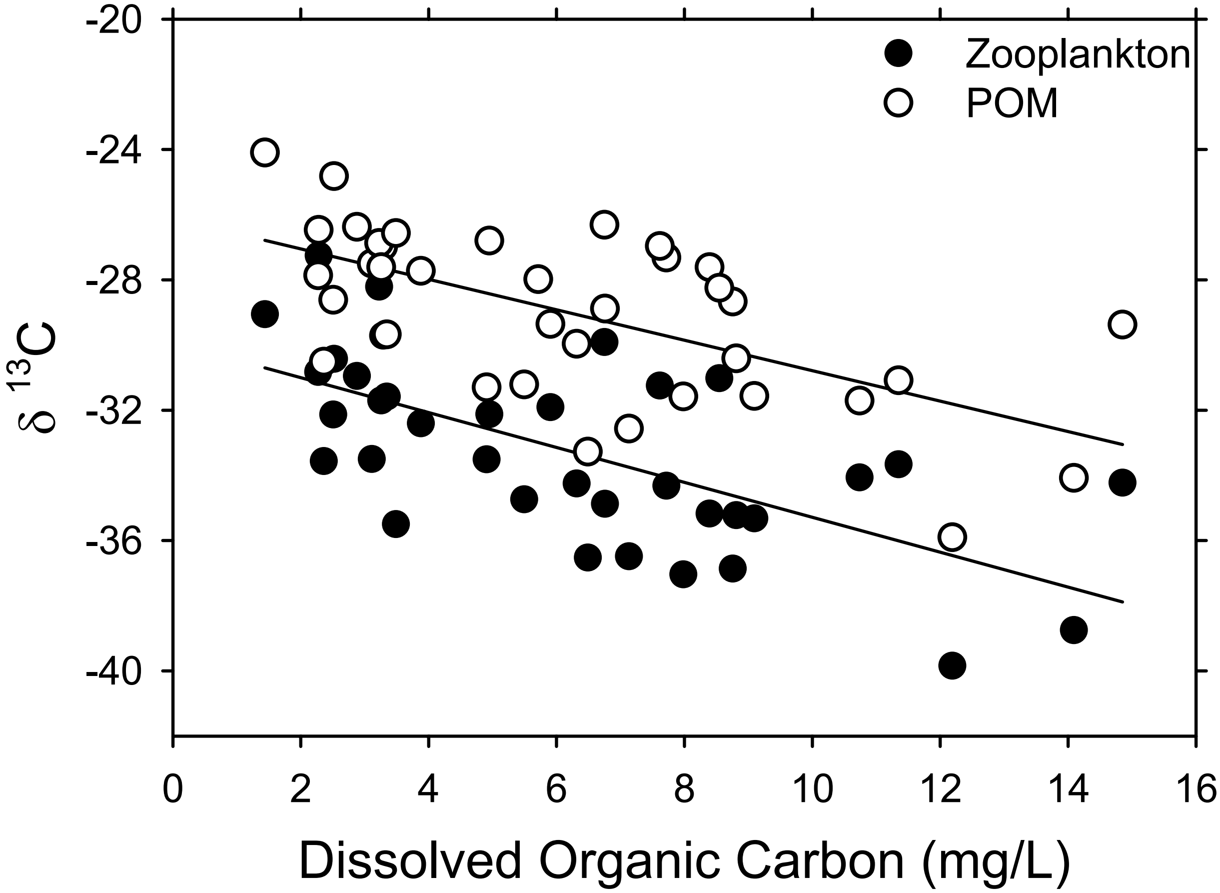

Figure 2. $\delta^{13}C$ of zooplankton (●) and POM (○) along a DOC gradient in 37 New England lakes. Multiple regression revealed that $\delta^{13}C$ of zooplankton and POM decreased at the same rate along the DOM gradient. However, the zooplankton intercept is significantly less than the POM source suggesting that POM does not perfectly reflect zooplankton diets.